PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 47082-149WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/US2004/035286	International filing date *(day/month/year)* 22.10.2004	Priority date *(day/month/year)* 24.10.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/00, C12Q1/26, G01N33/487, G01N27/403		
Applicant BAYER HEALTHCARE LLC et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.08.2005	14.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Stussi, E Telephone No. +49 89 2399-2265

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/035286

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-26 as originally filed

Claims, Numbers

1-73 received on 10.08.2005 with letter of 08.08.2005

Drawings, Sheets

1/11-11/11 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/035286

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-12,14-17,21,23-25,33,40-42,48-51,53,61-73
	No:	Claims	1-3,13,18-20,22,26-32,34-39,44-47,52,54-60
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-73
Industrial applicability (IA)	Yes:	Claims	1-73
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V.

1 The following documents are referred to in this communication:

D1: US-B1-6 287 451
D2: US 2001/006149
D3: EP-A-0 909 952
D4: MORRIS N A ET AL: "AN ELECTROCHEMICAL CAPILLARY..." ELECTROANALYSIS, vol. 4, no. 1, 1992, pp. 1-9, XP002065769
D5: VIDAL J C ET AL: "A chronoamperometric sensor for hydrogen peroxide ...", SENSORS AND ACTUATORS B, vol. 21, no. 2, (1994-08), pp.135-141, XP004012315
D6: RAZUMIENE J ET AL: "Improvement of screen-printed carbon electrodes by modification with ferrocene derivative" SENSORS AND ACTUATORS B, vol. 95, no. 1-3, (2003-10-15), pp. 378-383, XP004454696
D7: US-A-5 520 786

2 INDEPENDENT CLAIM 1

2.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 is not new in the sense of Article 33(2) PCT.
Document D1 discloses (the references in parentheses applying to this document):

an electrochemical sensor strip (claim 1, first line), comprising:
a base (20);
a first electrode (W2) on the base;
a first reagent layer on the first electrode, the first reagent layer comprising an electroactive organic molecule and an oxidoreductase capable of facilitating a redox reaction of a substrate (col.10, ll.49-52);
a second electrode (R) on the base; and
a second reagent layer on the second electrode, the second reagent layer comprising a first soluble redox species selected from the group consisting of an organotransition metal complex, a transition metal coordination complex and mixtures thereof, the first

soluble redox species being capable of undergoing a redox reaction opposite that of the substrate (col.10, ll.41-43, ferricyanide being an organotransition metal complex, as stated in the present application itself, cf. p.7, second paragraph).

2.2 Claim 1 is also not novel in view of documents D2 (cf. mediators listed in § 3 of D2, as compared to the list on p.7 § 2 and p.8 § 2 of the present application, whereby the fact that there is "preferably" no mediator on the working electrode does not exclude its presence) and D3 (cf. § 32, whereby the wording of claim 1 of the repsent application does not exclude the enzyme layer to be present on the second electrode as well).

3. INDEPENDENT CLAIM 30

Independent claim 30 is also anticipated by document D1, cf. in particular col.10, ll.52-53, disclosing the use of glucose oxidase, and col.10, l.55, disclosing the use of ferricyanide as a soluble redox species.

4. INDEPENDENT CLAIM 37

Independent claim 37 discloses no more than a standard method of producing the sensor strip of claim 1 and is also considered to be anticipated by D1 (Art. 33(2) PCT).

5. INDEPENDENT CLAIM 61

Independent claim 61 discloses the normal use of a sensor strip according to claim 1: its subject-matter does thus not involve an inventive step with respect to D1 (Art. 33(3) PCT).

6. DEPENDENT CLAIMS 2-29,31-36, 38-60 and 62-73

The above mentioned dependent claims do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, the reasons being as follows:

6.1 The additional features disclosed in claims 2, 3, 13, 18-20, 22, 26-29 (third electrode = electrode W1 of D1, cf. col.10, ll.41-44) 31, 32, 34-36, 38, 39, 44-47, 52 and 54-60 are known from D1 (Art. 33(2) PCT).

6.2 The additional features of claims 14 and 66 are not inventive in view of the combination of D1 with document D4 (Art. 33(3) PCT).

6.3 The additional features of claim 15, 24, 41, and 42 are not inventive in view of the combination of D1 with document D5 or D6 (Art. 33(3) PCT).

6.4 The additional features of claims 16, 17, 33 and 69 are not inventive in view of the combination of D1 with document D7 (Art. 33(3) PCT).

6.5 The additional features of claims 62-65, 68, and 70-73 are obvious in view of D1.

6.6 Claims 4-9, 12, 48, 49 (as far as these claims can be understood- see § VIII, points 3 and 4), 10-11, 21, 23, 25, 40, 43, 50, 51, 53, and 67 disclose additional features of the sensor strip or additional steps of the methods that are either obvious for the skilled man or do not appear to give raise to any unexpected technical effect. The subject matter of these claim is thus not inventive (Art. 33(3) PCT).

Re Item VII

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein. A similar objection stands for the documents D2-D7.

2. The features of the claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

3. The description is not adapted to the claims (Art. 6 PCT).

Re Item VIII.

1. The only difference between independent claim 30 and independent claim 1 is that claim 30 limits the scope of protection for the oxidoreductase of claim 1 to glucose oxidase or glucose hydrogenase. Therefore, since claim 30 contains all the features of claim 1 it should depend on it (R. 6.4 PCT).

2. Independent claims 37 and 61 relate to the manufacture and use of a strip according to claim 1 and should thus relate explicitly to this claim (Art. 6 PCT).

3. Claims 4, 5, and 12 do not meet the requirements of Art. 6 PCT: for the following reasons.
It is stated in the description (p.8, ll. 10-13) that an "electroactive organic molecule" can be a particular form of "mediator", and in fact the former wording has been substituted for the latter in new claim 1. At the same time old claim 2 stated that the "soluble redox species" can be an "electroactive organic molecule" (cf. also p.14, l.29).
In conclusion it appears that "mediator", "electroactive organic molecule" and "soluble redox species" are (or at least can be) different names for the same substances. Since claims 4, 5 and 12 appear to presuppose a difference between said names, said claims are unclear in view of the description.

4. Claims 6 (and consequently 7 to 9), and 48 (and consequently 49) do not meet the requirements of Art. 6 PCT because it is not clear what by "first" and "second" is meant. It is assumed that the claims disclose the presence of both the oxidised and reduced form of the same redox compound on the second electrode. However it is not clear which of the two oxidation forms is present in which quantity.

Claims 1-63 amended; claims 64-73 added

CLAIMS:

1. An electrochemical sensor strip, comprising:

a base;

a first electrode on the base;

a first reagent layer on the first electrode, the first reagent layer comprising an electroactive organic molecule and an oxidoreductase capable of facilitating a redox reaction of a substrate;

a second electrode on the base; and

a second reagent layer on the second electrode, the second reagent layer comprising a first soluble redox species selected from the group consisting of an organotransition metal complex, a transition metal coordination complex and mixtures thereof, the first soluble redox species being capable of undergoing a redox reaction opposite that of the substrate.

2. The electrochemical sensor strip of claim 1, wherein the composition of the first reagent layer is different from the composition of the second reagent layer.

3. The electrochemical sensor strip of claim 2, wherein the oxidoreductase is substantially present only in the first reagent layer.

4. The electrochemical sensor strip of claim 2, wherein the electroactive organic molecule is substantially present only in the first reagent layer.

5. The electrochemical sensor strip of claim 2, wherein the first soluble redox species is substantially present only in the second reagent layer.

6. The electrochemical sensor strip of claim 2, wherein the second reagent layer further comprises a second redox species, the second redox species being a species of a redox pair comprising the first soluble redox species and the second redox species, and wherein the molar ratio of the first soluble redox species to the second redox species is greater than 1.2:1.

7. The electrochemical sensor strip of claim 6, wherein the molar ratio of the first soluble redox species to the second redox species is greater than 2:1.

8. The electrochemical sensor strip of claim 7, wherein the molar ratio of the first soluble redox species to the second redox species is greater than about 10:1.

9. The electrochemical sensor strip of claim 6, wherein the second redox species is present in an amount less than 1 part per thousand.

10. The electrochemical sensor strip of claim 6, wherein the first soluble redox species has a standard reduction potential of at least +0.24 volts.

11. The electrochemical sensor strip of claim 10, wherein the first soluble redox species has a standard reduction potential of at least +0.35 volts.

12. The electrochemical sensor strip of claim 6, wherein the first reagent layer further comprises the second redox species.

13. The electrochemical sensor strip of claim 1, wherein the first soluble redox species comprises ferrocyanide or ferricyanide.

14. The electrochemical sensor strip of claim 1, wherein the first soluble redox species comprises ruthenium(II) hexaamine or ruthenium(III) hexaamine.

15. The electrochemical sensor strip of claim 1, wherein the electroactive organic molecule is selected from the group consisting of coenzyme pyrroloquinoline quinone (PQQ), substituted benzoquinones, substituted naphthoquinones, N-oxides, nitroso compounds, hydroxylamines, oxines, flavins, phenazines, phenothiazines, indophenols, indamines, phenazinium salts, phenoxazinium salts, 3-phenylimino-3H-phenothiazines, 3-phenylimino-3H-phenoxazines, and mixtures thereof.

16. The electrochemical sensor strip of claim 1, wherein the electroactive organic molecule comprises a 3-phenylimino-3H-phenothiazine.

17. The electrochemical sensor strip of claim 1, wherein the electroactive organic molecule comprises a 3-phenylimino-3H-phenoxazine.

18. The electrochemical sensor strip of claim 1, wherein the oxidoreductase is selected from the group consisting of glucose dehydrogenase, glucose oxidase, cholesterol esterase, cholesterol oxidase, lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, lactate oxidase, lactate dehydrogenase, diaphorase, pyruvate oxidase, alcohol oxidase, bilirubin oxidase, uricase, glutathione reductase, and carbon monoxide oxidoreductase.

19. The electrochemical sensor strip of claim 1, wherein the oxidoreductase comprises an enzyme selected from the group consisting of an oxidase and a dehydrogenase, and wherein the first soluble redox species is a reducible species.

20. The electrochemical sensor strip of claim 19, wherein the oxidoreductase comprises glucose oxidase or glucose dehydrogenase.

21. The electrochemical sensor strip of claim 1, wherein the oxidoreductase comprises a reductase, and wherein the first soluble redox species is an oxidizable species.

22. The electrochemical sensor strip of claim 1, further comprising a lid.

23. The electrochemical sensor strip of claim 1, wherein the second electrode comprises a surface layer comprising a non-ionizing conducting material.

24. The electrochemical sensor strip of claim 23, wherein the surface layer comprises carbon.

25. The electrochemical sensor strip of claim 1, wherein the second electrode comprises a non-ionizing conducting material.

26. The electrochemical sensor strip of claim 1, further comprising a third electrode on the base and a third reagent layer on the third electrode, the third reagent layer comprising a third soluble redox species.

27. The electrochemical sensor strip of claim 26, wherein the third soluble redox species is substantially identical to the first soluble redox species.

28. The electrochemical sensor strip of claim 2, further comprising a third electrode on the base and a third reagent layer on the third electrode, the third reagent layer comprising a third soluble redox species.

29. The electrochemical sensor strip of claim 28, wherein the third soluble redox species is substantially identical to the first soluble redox species.

30. An electrochemical sensor strip, comprising:

a base;

a first electrode on the base;

a first reagent layer on the first electrode, the first reagent layer comprising an electroactive organic molecule and an enzyme selected from the group consisting of glucose oxidase, glucose dehydrogenase, and mixtures thereof;

a second electrode on the base; and

a second reagent layer on the second electrode, the second reagent layer comprising a first soluble redox species selected from the group consisting of an organotransition metal complex, a transition metal coordination complex, and mixtures thereof, the first soluble redox species being a reducible species.

31. The electrochemical sensor strip of claim 30, wherein the composition of the first reagent layer is different from the composition of the second reagent layer.

32. The electrochemical sensor strip of claim 31, wherein the electroactive organic molecule and the enzyme are substantially present only in the first reagent layer, and the first soluble redox species is substantially present only in the second reagent layer.

33. The electrochemical sensor strip of claim 30, wherein the electroactive organic molecule is selected from the group consisting of 3-phenylimino-3H-phenothiazines, 3-phenylimino-3H-phenoxazines, and mixtures thereof.

34. The electrochemical sensor strip of claim 30, wherein the first soluble redox species is selected from the group consisting of ferricyanide and ruthenium(III) hexaamine.

35. The electrochemical sensor strip of claim 30, further comprising a third electrode on the base, and a third reagent layer on the third electrode, the third reagent layer comprising a third soluble redox species.

36. The electrochemical sensor strip of claim 30, wherein the third soluble redox species is substantially identical to the first soluble redox species.

37. A method of making an electrochemical sensor strip, the method comprising the acts of:

depositing a first electrode on a base;

depositing a second electrode on the base;

applying a first reagent layer on the first electrode, the first reagent layer comprising an electroactive organic molecule and an oxidoreductase capable of facilitating a redox reaction of a substrate; and

applying a second reagent layer on the second electrode, the second reagent layer comprising a first soluble redox species selected from the group consisting of an organotransition metal complex, a transition metal coordination complex, and mixtures thereof, the first soluble redox species being capable of undergoing a redox reaction opposite that of the substrate.

38. The method of claim 37, wherein the oxidoreductase comprises an enzyme selected from the group consisting of an oxidase and a dehydrogenase, and wherein the soluble redox species is a reducible species.

39. The method of claim 38, wherein the oxidoreductase comprises glucose oxidase or glucose dehydrogenase.

40. The method of claim 37, wherein the oxidoreductase comprises a reductase, and wherein the soluble redox species is an oxidizable species.

41. The method of claim 37, wherein the depositing the first electrode comprises screen printing a pattern of conductive carbon.

42. The method of claim 41, wherein the depositing the second electrode comprises screen printing a pattern of conductive carbon.

43. The method of claim 37, wherein the depositing the second electrode comprises depositing a pattern of a non-ionizing conductive material.

44. The method of claim 37, further comprising covering a portion of the base with a dielectric layer such that the first and second reagent layers are exposed.

45. The method of claim 37, further comprising mating a lid to the base such that the lid is over the first and second electrodes and the first and second reagent layers.

46. The method of claim 37, wherein the composition of the first reagent layer is different from the composition of the second reagent layer.

47. The method of claim 46, wherein the electroactive organic molecule and the oxidoreductase are substantially present only in the first reagent layer, and the first soluble redox species is substantially present only in the second reagent layer.

48. The method of claim 46, wherein the second reagent layer further comprises a second redox species, the second redox species being a species of a redox pair comprising the first soluble redox species and the second redox species, and wherein the molar ratio of the first soluble redox species to the second redox species is greater than 10:1.

49. The method of claim 48, wherein the second redox species is present in the second reagent layer in an amount less than 1 part per thousand.

50. The method of claim 48, wherein the first soluble redox species has a standard reduction potential of at least +0.24 volts.

51. The method of claim 50, wherein the first soluble redox species has a standard reduction potential of at least +0.35 volts.

52. The method of claim 46, wherein the applying the first reagent layer comprises dispensing a first aqueous composition comprising the oxidoreductase and the electroactive organic molecule.

53. The method of claim 52, wherein the first aqueous composition further comprises a redox cofactor for the oxidoreductase.

54. The method of claim 52, wherein the first aqueous composition further comprises a binder.

55. The method of claim 52, wherein the first aqueous composition further comprises a buffer.

56. The method of claim 46, wherein the applying the second reagent layer comprises dispensing a second aqueous composition comprising the first soluble redox species.

57. The method of claim 56, wherein the second aqueous composition further comprises a buffer.

58. The method of claim 37, further comprising depositing a third electrode on the base, and applying a third reagent layer on the third electrode, the third reagent layer comprising a third soluble redox species.

59. The method of claim 58, wherein the third soluble redox species is substantially identical to the first soluble redox species.

60. The method of claim 59, wherein the composition of the third reagent layer is substantially identical to the composition of the second reagent layer.

61. A method of quantifying an analyte in a sample, the method comprising the acts of:

contacting the sample with an electrochemical sensor strip, the electrochemical sensor strip comprising a first electrode and a first reagent layer on the first electrode, the first reagent layer comprising an electroactive organic molecule and an oxidoreductase capable of facilitating a redox reaction of a substrate, the electrochemical sensor strip further comprising a second electrode and a second reagent layer on the second electrode, the second reagent layer comprising a soluble redox species selected from the group consisting of an organotransition metal complex, a transition metal coordination complex, and mixtures thereof, the soluble redox species being capable of undergoing a redox reaction opposite that of the substrate;

applying an electrical potential between the first and second electrodes;

measuring a current passing through the first and second electrodes and the sample; and

correlating the current to a concentration of the analyte.

62. The method of claim 61, wherein the soluble redox species solubilizes in the sample and mixes with the electroactive organic molecule and an oxidoreductase.

63. The method of claim 61, wherein the analyte is the substrate for the oxidoreductase.

64. The method of claim 61, wherein the analyte is a cofactor for the oxidoreductase.

65. The method of claim 61, wherein the oxidoreductase comprises an enzyme selected from the group consisting of an oxidase and a dehydrogenase, the soluble redox species being a reducible species.

66. The method of claim 65, wherein the soluble redox species is selected from the group consisting of ferricyanide and ruthenium(III) hexaamine.

67. The method of claim 61, wherein the oxidoreductase comprises a reductase, and wherein the soluble redox species is an oxidizable species.

68. The method of claim 61, wherein the analyte comprises glucose, and the oxidoreductase comprises glucose oxidase or glucose dehydrogenase.

69. The method of claim 61, wherein the electroactive organic molecule is selected from the group consisting of 3-phenylimino-3H-phenothiazines, 3-phenylimino-3H- phenoxazines, and mixtures thereof.

70. The method of claim 61, wherein the electrochemical sensor strip further comprises a third electrode comprising the soluble redox species.

71. The method of claim 70, wherein the third electrode measures a second electrical potential between the third electrode and the first electrode, and the measured second electrical potential is used to adjust the electrical potential between the first and second electrodes.

72. The method of claim 61, wherein the composition of the first reagent layer is different from the composition of the second reagent layer.

73. The method of claim 72, wherein the electroactive organic molecule and the oxidoreductase are substantially present only in the first reagent layer, and the soluble redox species is substantially present only in the second reagent layer.